# CONTRIBUTIONS FROM THE CRYPTOGAMIC LABORATORY OF HARVARD UNIVERSITY. XLIV.

## NEW OR LITTLE KNOWN UNICELLULAR ALGÆ. I. CHLOROCYSTIS COHNII.

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(WITH PLATE X)

EVER since the discovery by Cohn, in 1872, of the chlorophyllous endophyte, *Chlorochytrium Lemnæ*, there has been considerable interest in algæ having such a habit, and much speculation has been indulged in, both as to their affinities and the method whereby they acquired their peculiar condition.

Probably the first recorded instance of a green alga living within the tissues of a host was that described by Mettenius (13) in 1850. This author found that in the fronds of *Polyides rotundus* (Gmelin) Grev. were curious green cells which were more or less crowded together and completely surrounded by the substance of the alga. These cells Mettenius considered to be the mother cells of the spores of Polyides, but Cohn (1) thought it more likely that they were young plantlets of some grass-green alga. Thuret (1) in a letter to Cohn confirmed this view and identified the plant as *Cladophora lanosa* (Roth) Kütz. He explains that he found the germinating zoospores within the cortical tissues of the Polyides, which gradually increased in size without dividing. Towards the end of the winter, however, they elongated rapidly, breaking through the tissues of the Polyides, and developed into small Cladophora tufts. Cohn found similar plants at Heligoland, but did not observe that they became septate or grew through the tissues of the host plant; hence he regarded them as being distinct endophytes. Some years previous to this, Cohn (2) described some long, narrow, green cells which occurred among the densely packed filaments of *Petrocelis cruenta* J. Ag. He at first regarded these as the normal reproductive cells of the Petrocelis, but later came to consider them as something quite separate from this plant. These green cells growing with Petrocelis have been found in this country by Dr. Farlow (6), and recently Kuckuck (11) has decided the plant to be a Codiolum, having no distant connection with its supposed host.

After Chlorochytrium was described on Lemna a number of endophytic forms were discovered, some of which showed such marked resemblances to certain fungi that, had it not been for their green color, they would undoubtedly have been placed within that group. One of these "green parasites," as they were popularly termed, was found by Wright (17) in 1876 growing on various algæ off the coast of Ireland, and called by him *Chlorochytrium Cohnii*. The discoverer of this form was so impressed with its fungus-like appearance and habit that he devoted considerable space to the discussion of how the plant was in reality a fungus which had but recently acquired the property of manufacturing chlorophyll. He was even able to observe the stages in this process as the plant developed. It is not my intention to go into a discussion of how fungi and endophytic algæ are related to each other; I merely wish to describe one of the algal forms, and any comparisons to be made with the fungi must be left to another time.

While collecting along the beach at Lynn, Mass., in February 1897, my attention was attracted to the peculiar granular appearance of some Enteromorpha which was growing attached to piles. When brought into the laboratory and examined under the microscope, the alga was seen to be covered with a green unicellular organism which at first did not seem to have been previously described. Upon more careful examination and an exhaustive search of the literature upon the subject, it was thought that this plant must be the endophytic alga found by Wright, *Chlorochytrium Cohnii*. The material collected by me, however, did not perfectly agree with any published account of this species, and according to the keys in both De Toni (3) and Engler and Prantl (5) could not find a place within that genus.

It seemed necessary, therefore, to study the structure and development of the plant more carefully before it could be decided whether or not it really was a new genus.

Since the plant was discovered by Wright, there have been but three published accounts of this form, for, although it occurs in widely separated regions and upon a number of different hosts, it is but rarely collected. Lagerheim (12) in 1884 found it off the coast of Sweden, and the next year Reinhardt (14) came upon it near Sebastopol, while investigating the flora of the Black Sea. The latter observer considered that the variations in this form from that of the original Chlorochytrium described by Cohn were sufficient to place it within a new genus, and he consequently proposed the name *Chlorocystis* which it has since borne. In 1892 de Wildeman (4) secured material from off the coast of France and published a short account of the plant. It has been reported from Greenland by Rosenvinge (15) and a new species growing on *Sarcophycus potatorum* has been described by Miss Whitting (16).

The material collected at Lynn frequently showed the Enteromorpha to be so nearly covered by the Chlorocystis as to cause it to appear rough and somewhat distorted even with a hand lens (*figs. 1* and *2*). The smaller more delicate pieces of the host plant seemed to be a more favorable resting place for the unicellular alga, and it was only occasionally that an individual was found upon the larger more exposed plants. The only reason discernible for this was the fact that the Enteromorpha was exposed to the air except at high tide, and the smaller fronds, growing in tufts and more closely adhering to the piles, retained the moisture longer and were consequently more favorable for growth.

When viewed with the ordinary low powers of a microscope, Chlorocystis appears a bright green color, usually of exactly the same shade as the Enteromorpha cells, but is not easily mistaken for them. If the cells of the host plant are dividing to form branch-like outgrowths and project above the surrounding tissue, there is a slight resemblance to the young

Chlorocystis, but a careful examination will at once reveal the difference.

In shape *Chlorocystis Cohnii* is usually spherical, although it may be slightly elliptical. It measures from 16–26$\mu$ in the mature condition. Even though the plants are frequently crowded together in irregular masses, they never lose their characteristic outline.

The question as to the degree to which Chlorocystis may infest the host plant is one upon which my observations do not agree with those made previously. Both Lagerheim (**12**) and de Wildeman (**4**) describe the plant as being completely surrounded by the cells of the host, except for a small colorless portion which projects beyond the surface, through which the zoospores escape. Wright in his original description conveys the same idea, although he says, "sometimes zoospores attach themselves in such quantities to Schizonema that there is no room to force themselves into the frond," and at such times they are said to show but little evidence of penetrating the host. *Chlorocystis sarcophyci* (**16**) is described as being completely embedded within the tissue of Sarcophycus. As may be seen from *fig. 2*, the plants as I found them were not always included within the host, but were quite as often merely attached to the surface of the Enteromorpha. While, as will be described later, the zoospores upon germination may send out processes which penetrate between the Enteromorpha cells and during further development may be more or less surrounded by these cells, the fact remains that many of the plants pass their entire existence without having at any time been within the tissues of the host. The crowded condition of which Wright speaks is not necessary to bring this about, for the epiphytic habit is just as apt to occur among single individuals entirely separated from one another, as when they are grouped together. Even when the lower half of the Chlorocystis cell is below the Enteromorpha (*fig. 5, b*), a firm but gentle pressure will usually free it, leaving a round clear space where it has crowded the host cells apart. At no time were cells observed completely covered by the Enteromorpha, and the impossibility of such an occurrence is easily understood when we remember that the single layer of Enteromorpha cells is frequently less than half the size of the Chlorocystis, and that they could never any more than surround the alga in a very superficial way. When the host is a plant made up of a mass of tissue, it may be that Chlorocystis assumes a true endophytic habit, but in Enteromorpha it certainly does not seem possible. Occasionally zoospores get between the tubular frond of the host through some accidental opening and there develop into normal Chlorocystis cells, just as they would on the surface of the plant, but this can hardly be called endophytic.

There is no evidence that the Enteromorpha is inconvenienced in any serious way by the presence of the Chlorocystis. Even when the fronds were almost completely covered, the cells of the host plant retained their normal appearance and seemed to be capable of carrying on all their functions. *Chlorocystis sarcophyci*, according to Miss Whitting (16), exerts a direct influence upon the surrounding tissue, "causing at first a swelling and loosening of the tissue, and finally complete disintegration of the cells," but nothing of the kind takes place with *Chlorocystis Cohnii.* The only benefit the Chlorocystis seems to derive from its host is that of a convenient and easy place of attachment, the condition well expressed by the German term *Raumparasitismus.* The algæ upon which *Chlorocystis Cohnii* have been found are quite various. *Enteromorpha* and *Urospora* among the Chlorophyceæ; *Polysiphonia* among the Rhodophyceæ, *Ascophyllum* among the Phæophyceæ, and *Navicula* (*Schizonema*) among the Diatomaceæ have all been recorded as host-plants for this form. The hydrozoan, *Campanularia*, and the infusorian, *Vaginicola*, have also been found with Chlorocystis growing upon them, and it seems not unlikely that the peculiar condition described as the abnormal fruit of *Calothrix confervicola* by Harvey (7) and the abnormal cells of *Prasiola leprosa* figured by Jessen (8) may likewise be due to this alga.

Chlorocystis contains a single large chromatophore, which, according to Reinhardt (14), and Wille in Engler and Prantl (5), is said to lie always upon but one side of the cell. This is not true, however, and it was only after a considerable number of specimens were examined that the "one-sided" chromatophore was observed. It so happened that all the specimens which were first found showed a chromatophore completely lining the cell wall, as indicated in *figs. 1, 2,* and *4*. Later, however, examples were found more nearly resembling Reinhardt's figures (*figs. 3, 5a*); but while this condition frequently occurs, it can no more be considered characteristic than when the whole cell is lined. A large and easily discernible pyrenoid lies near the surface of the cell and can be followed through all the subsequent divisions of the chromatophore (*figs. 6, 7*). Material killed in picric acid and stained for some time in 2 per cent. acid fuchsin brought out the pyrenoids well, although Flemming's fluid with iron-alum-haematoxylin gave perhaps more satisfactory results. The chromatophore usually forms a definite dome-like thickening where it surrounds the pyrenoid, and this may extend into the cell in the way shown in *fig. 5a*. When the chromatophore does not entirely line the wall, it radiates from the pyrenoid in irregular bands or ribbons, and these frequently do not pass more than half way round the cell (*fig. 5a*). This is the condition which Reinhardt figures and which he considered a generic characteristic. It was thought for a time that cells in which the chromatophore formed a complete lining might represent a condition subsequent to the formation of zoospores and not really be the adult Chlorocystis. Cultures in a Van Tieghem cell did not give much information on this subject, for although zoospores would be formed and escape, they did not develop to anywhere near maturity. This was probably due to an insufficient supply of oxygen, for when cultures were made in Ward cells, or simply under large cover glasses kept in a moist chamber, the zoospores could be watched from the time they escaped until they attained their full development. By this means it was settled beyond a doubt that in the great majority of cases the chromatophore entirely lined the cell from the beginning, and that it was a perfectly normal arrangement throughout all the vegetative stages of the plant.

Near the center of the cell is a well-defined nucleus, from which the protoplasm radiates in fine strands. There are usually several small non-contractile vacuoles present and the whole cell contents is often quite granular.

Two sizes of zoospores are formed. The larger ones, which are spherical, measure 6–7 $\mu$ in diameter (*figs. 10, 12*), while the smaller are only 2.6–3.5 $\mu$ and are somewhat pyriform in outline (*figs. 11, 13*). The method of the formation of the zoospores is identical in both cases, except that there are more successive divisions in the formation of the smaller spores, thus producing a greater number. In the original description of the genus by Wright, it is stated that the zoospores are formed in a very few hours by free cell formation. By Reinhardt (**14**) this simultaneous formation is considered one of the points of distinction between Chlorocystis and Chlorocytrium. De Toni also uses this distinction to separate the two genera. That the zoospores in Chlorocystis are formed by free cell formation is undoubtedly wrong, and all of my observations go to substantiate those of Lagerheim (**12**) and de Wildeman (**4**), who both state that the spores are formed by successive divisions. All stages in this process may be observed (*figs. 6, 7, 9*), and nothing comparable to the description by Wright has ever been seen. Also the statement made by the discoverer of the genus that the zoospores are at first colorless and that the protoplasm seemed to project itself to one pole and there form a single cilium is not borne out by my observations. Both kinds of zoospores have each four cilia, with a single chromatophore lining the base of the cell. In the large zoospores the pyrenoid is easily made out, and in the hyaline end of both the large and small spores is found a lenticular or spherical red spot.

When the zoospores are fully formed and ready to escape, a circular piece about 10 $\mu$ in diameter is cut out from the top or outer side of the cell. This may be entirely loosened, or frequently it simply turns back, remaining attached at one side (*fig. 11*) very much as in some of the Chytridineæ. It seems probable that the zoospores do not always escape in this manner, for all the other observers of this plant have spoken of a colorless neck which projects beyond the surface of the cell and through which the zoospores make their way. Wright, however, has said to Miss Whitting (16) that when he found *Chlorocystis Cohnii* developed in the interior of tissue, the cells were sometimes quite globular. Certainly the figures of Lagerheim (12) and Reinhardt (14) do not correspond to the description of a plant possessing such a protuberance. If the *Chlorochytrium inclusum* of Kjellmann (9) is finally to find a place within the genus Chlorocystis, as has been suggested, we have still another example of a form without the neck-like protuberance. After the examination of dried specimens of *Chlorochytrium inclusum* Kjell. I am not inclined to think that it is a Chlorocystis; although the published figures are strongly suggestive of that genus. It is certain that at no time, among the hundreds of specimens of Chlorocystis which were examined, was there anything that resembled a colorless protuberance. It may be that the varying habitat has something to do with the difference in aspect which this alga often presents; at any rate it seems probable that the presence or absence of a colorless tubular portion through which the zoospores may escape is not of much importance.

When the zoospores are liberated they swim about for a length of time varying from a few minutes to two hours. No difference was discernible in the rate or length of activity of the two kinds of spores. In almost every case the spores escaped perfectly free and independently of each other, but in a very few instances it appeared as though they might have been enclosed in a delicate membrane as in Chlorochytrium. If there was such a membrane it must have been very frail and was suggested rather by the arrangement of the zoospores than by any actual observation. It always seemed to break up before any reagent could be added to demonstrate it, and it is quite possible that nothing of the kind exists. Such a membrane enclosing the zoospores could not be of any significance from a systematic standpoint, for even in forms where it occurs frequently, there are conditions which bring about its total disappearance.

Material in the laboratory showed the time for the escape of the zoospores to be usually from seven to ten o'clock in the morning. This probably varies with the changing conditions at the seashore, and since the alga was submerged for only a few hours twice a day, it seems likely that the time of zoospore discharge varies with the tides. Efforts to establish this fact were unavailing. Observations made during the night were likewise without result. Perfect aeration was found to be conducive to the formation and discharge of large numbers of zoospores.

The existence of two kinds of zoospores and the fact that conjugation takes place in certain closely related genera would naturally lead to the supposition that something of the same kind occurs in Chlorocystis. De Wildeman (4) quotes Lagerheim as having observed copulation, but I am unable to find such a statement in any of Lagerheim's papers. He does mention having seen two kinds of zoospores, and considers it probable that the larger spore is formed by conjugation, but I think does not claim to have seen the process. From my own observations I can say that it is certain the larger zoospores are not formed by conjugation, and that it is possible for both sizes of zoospores to develop into new plants without any fusion. This point was carefully investigated by means of Van Tieghem cell and other cultures, and the zoospores were observed during their escape and final coming to rest. There was at no time any appearance of conjugation, and the development of the spores, whether of the large or small variety, was always the same, the cells produced being similar in every particular to the characteristic adult plants. It may be that under different physiological conditions conjugation might occur, but at the present time no light can be thrown upon that point.

When a zoospore comes to rest upon the surface of the host-plant, its cilia disappear and a thin gelatinous wall is formed around it. The red spot is lost to view and the pyrenoid becomes more prominent. If the zoospore is to develop within the host instead of merely attaching itself to the surface, a short colorless neck is pushed out, and this penetrates the Enteromorpha frond between its cells and pushes them apart. When an entrance has been gained in this manner, the neck widens until the whole cell appears funnel-shaped, and this, after further growth, assumes its mature spherical condition. In a few instances zoospores were observed which had germinated without having come in contact with the host-plant, and it is an interesting fact that some of these sent out colorless tubes of a considerable length (*fig. 8*). These were all found in cultures of various kinds and may have been due to some unknown abnormal condition.

In the first published account of this plant, the zoospores were described as escaping from the mother cell without possessing any color. These colorless zoospores developed into colorless plants which remained so until they had nearly reached adult size when the protoplasm commenced to develop "green cromules." "These cromules," says Wright (**16**), "arise as minute points along the inner surface of the cell wall from whence they radiate to the nucleus giving the appearance of a number of necklaces hung in loops." Although I looked carefully for some such condition in my material I was unable to observe anything abnormal or unusual. The green zoospores gradually developed into mature green plants with definite chromatophores as described.

Resting spores were observed in material that had been kept in the laboratory for some time and had been allowed to dry up partially. They are formed by the thickening of the wall of the mature plant and the contents rolling itself into a solid mass of irregular outline. The spore thus becomes of a darker green shade, and the pyrenoid is lost in the increased density of the cell contents.

It will be seen from the foregoing that a number of points with regard to the structure and development of *Chlorocystis Cohnii* which have been considered by former investigators as characteristic can no longer remain as such. The habit of the plant is variable, and it certainly cannot be regarded as a universal endophyte. The chromatophore is quite as apt to line the cell wall as to be confined to one side and the radiate arrangement of the coloring matter may or may not occur. The method of zoospore formation is certainly the same as described by Klebs (10) for *Chlorochytrium Lemnæ*, namely by successive division. Even the manner in which the zoospores escape seems to vary, and the presence or absence of the colorless tube is of but little consequence.

It may be questioned whether or not the material found at Lynn really was Chlorocystis, since it fails to agree with any published account. Certainly much the easier way would be to regard it as a new genus. But while the plants found do not agree with the keys in de Toni, and Engler and Prantl, or with any other published account, the points of resemblance are very marked when all the literature is considered as a whole and the various generic characteristics correlated. The few papers on the subject are strangely at variance, and the figures in at least one case do not agree at all with the accompanying description, nor with the specimens distributed by the author. Consequently it seems a case where we are justified in disregarding certain published accounts and in considering that the form above described is really what Wright and Reinhardt meant for the plant *Chlorocystis Cohnii.*

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## EXPLANATION OF PLATE X.

All the figures are from ink drawings sketched in with an Abbé camera. In the reproduction they are reduced one fourth. *Figs. 1* and *2* are drawn with a Leitz $\frac{1}{5}$, oc. 3. All the others with a Leitz $\frac{1}{12}$ (oil), oc. 3. The magnifications given are the original ones before reduction and allow for projection.

*Chlorocystis Cohnii* (Wright) Reinhardt.

FIG. 1. General habit showing appearance of cells in Enteromorpha frond. × 280.

FIG. 2. Section through Enteromorpha showing relation of Chlorocystis to its host. × 280.

FIG. 3. Surface view of single cell. "One-sided" chromatophore with pyrenoid and radiate arrangement. × 830.

FIG. 4. Surface view of single cell with chromatophore lining entire wall. × 830.

FIG. 5. Side view; *a*, showing arrangement of radiating "one-sided" chromatophore; *b*; zoospore which has come to rest directly over Enteromorpha

cell; *c*, developing zoospore with projecting neck penetrating between Enteromorpha cells. × 830.

FIGS. 6, 7. Surface views of first two stages in the formation of zoospores. × 830.

FIG. 8. Germinating zoospores in cultures not in contact with host. × 830.

FIGS. 9, 10. Sporangia of large and small zoospores respectively. × 830.

FIG. 11. Sporangium of large zoospores showing method of discharge. × 830.

FIGS. 12, 13. Large and small zoospores. × 830.

FIG. 14. Resting spore.